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Investigation by Linkage Analysis of the XY Pseudoautosomal Region in the Genetic Susceptibility to Schizophrenia

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Background. A susceptibility locus for schizophrenia in the pseudoautosomal region has been proposed on the basis of a possible excess of sex chromosome aneuploidies among patients with schizophrenia and an increased sex concordance in affected sib pairs. Several studies investigating this hypothesis have produced conflicting evidence.
Method. In a series of Icelandic and British families, we used lod score and sib pair linkage analyses with markers for the MIC2 and DXYS14 loci on the pseudoautosomal XY region.
Results. Lod and sib pair linkage analysis with these markers produced strongly negative scores. Heterogeneity testing also produced negative results.
Conclusion. We conclude that the present study provides no support for the involvement of either the pseudoautosomal region or the nearby region of the sex chromosomes in the aetiology of schizophrenia.

By definition, genes located in the pseudoautosomal region are transmitted in an autosomal rather than a sex-linked manner and characterised by certain features. For example, a single obligatory cross-over occurs in each male meiosis and there is a differential recombination rate of 3% in females and 50% in males in the pseudoautosomal region during meiosis. The possibility that genes determining sex might play a role in the cause of mental illness was first suggested by Penrose (1942) after considering the possibility that a pseudoautosomal location of the psychosis gene would explain the observed increased rate of same-sex concordance in affected siblings. Evidence for an increased incidence of male/male schizophrenic pairs was presented by Crow (1988). An increased risk of aneuploidies of the sex chromosomes has been observed, and although most cases of sex-chromosomal aneuploidy are not associated with schizophrenic illness, the association exceeds expectation, which suggests a causal relationship with sex chromosome genes.

There have been a number of recent attempts, using various types of analyses, to test the possibility of sex chromosome involvement. Collinge *et al* (1991) used sib pair analysis with the pseudoautosomal marker DXSY14, and concluded that there was evidence for a schizophrenia locus in the vicinity. Asherson *et al* (1992) studied Welsh and English families for sex concordance and although an excess of same-sex affected pairs was found within the sample, this was not statistically significant, so that the authors could not conclude in favour of the pseudoautosomal locus. However, studies by D'Amato *et al* (1992) and Gorwood *et al* (1992) carried out in the manner of Asherson *et al* (1992) on 38 French multiplex families did show an excess of same-sex male pairs of patients. Like Asherson *et al* (1992), Barr *et al* (1994) and Wang *et al* (1993) found no evidence of a pseudoautosomal locus for schizophrenia; nor did the recent study by Crow *et al* (1994) support pseudoautosomal transmission. When Crow *et al* allowed recombination fraction to vary independently in males and females, they obtained a lod score of 2.44 with MIC2, which is near the boundary of the pseudoautosomal region. This occurred with a high female:male recombination ratio, and on this basis the authors postulated a locus for schizophrenia on the other side of the pseudoautosomal boundary, in the sex-specific regions of the X and Y chromosomes. The hypothesis was tested further by checking allele sharing of maternal alleles. The results were not statistically significant, and the authors suggested that transmission is paternal, with the possibility that the positive lod score at MIC2 may be due to linkage on the Y chromosome. In our view, the weakly positive lod scores may have occurred as a result of using a sample containing an excess of affected males. Since the study uses markers linked to sex, this would inevitably produce artefactual evidence in favour of linkage when there is an excess of affected sib pairs who are concordant for sex (Curtis *et al*, 1994). Like some of the previous studies, we have also employed the lod score and sib pair linkage

methods to test the pseudoautosomal hypothesis, by using the marker DXYS14, which is the most telomeric marker in the pseudoautosomal region, and MIC2 (probe p26C1), which is close to the boundary of the XY pseudoautosomal region. Like Crow *et al* (1994) we incorporated the use of 'sex' as a marker.

Methods

Altogether 23 pedigrees containing multiple cases of schizophrenia were studied. The basis for inclusion of pedigrees was that they should contain multiple cases of schizophrenia but no cases of bipolar affective illness, and should appear to demonstrate unilineal inheritance. Three affection classes were used for the linkage analyses: 'schizophrenia and nonaffective psychosis model' (denoted DOMS), consisting of schizophrenia and unspecified functional psychosis; 'schizophrenia spectrum' (DOMSS), consisting additionally of schizoid and schizotypal personality disorder; and 'schizophrenia fringe' (DOMSSF), consisting of any psychiatric diagnosis. Of the 385 individuals in the 23 pedigrees, 95 fell into the DOMS category, an additional 17 were DOMSS and a further 49 were DOMSSF.

DNA was extracted directly from frozen blood samples. 5 μg of DNA were digested to completion with an excess of *Taq*1 with the addition of 1 mM spermidine, size-fractionated by electrophoresis in 0.8% agarose gels, denatured for 1 h in 0.5 mol/l NaOH, 1.5 mol/l NaCl and transferred to nylon membranes (Hybond N, Amersham) in 20 × SSC (0.3 mol/l sodium citrate, 3 mol/l NaCl). The DNA was covalently linked to the membrane by UV irradiation for 2 min.

The probe p29C1, localised to the telomeric pseudoautosomal locus DXYS14, detects hypervariable (VNTR) fragments on Southern blots prepared from *Eco*R1 or *Taq*1-digested DNA. The probe p19B, localised to the more centromeric locus MIC2, detects restriction fragment length polymorphisms (RFLPs) on Southern blots prepared with *Taq*I or *Pvu*II digested DNA. *Taq*I filters were prehybridised at 65°C in 20 ml of 0.9 M NaCl, 1% SDS and 50 μg/ml of autoclaved denatured salmon testes DNA, for a minimum of 4 h. Hybridisation was carried out overnight at 65°C in 20 ml of fresh solution including 10% (w/v) dextran sulphate was well as 100 ng denatured oligolabelled probe using α-^{32}P at 2×10^6 cpm/ml. The membranes were then washed to a stringency of 1 to 0.1 × SSC, 0.1% SDS at 65°C. The filters were exposed against autoradiographic film at −70°C using intensifying screens.

Linkage analyses were carried out using MLINK and LINKMAP. The penetrance values for those heterozygous for the disease allele were specified as 0.73, 0.76 and 0.86 for the DOMS, DOMSS, and DOMSSF models respectively. Sporadic cases were accounted for by assuming a penetrance of 0.005, 0.01 and 0.05 respectively for the normal homozygote. The gene frequency of the abnormal allele was set to 0.0085. When calculating multipoint lod scores for estimation of linkage, 'sex' was used as a marker of the pseudoautosomal boundary. In order to test for a locus further down on the sex chromosomes, linkage analysis was repeated using MIC2 with the female to male distance ratio set to 10 (in order to reflect the unequal recombination rates in males and females). Analyses were also performed using the ILINK program to find the maximum lod score without any constraint on the female:male ratio.

In order to investigate the possibility that only a subset of pedigrees might have a susceptibility locus in the region studied, heterogeneity testing was carried out using the A-test procedure. We also employed the sib pair linkage programme ESPA (Extended Sib Pair Analysis) which computes a classical identity-by-descent sib pair analysis as well as an 'extended' sib pair analysis which can make use of pedigree information to infer missing genotypes.

Results

The two-point lod scores were calculated between the markers, sex and schizophrenia. At θ of zero the lod score between sex and MIC2 is about 7.8, confirming that MIC2 is strongly linked to sex. According to our data the marker DXYS14 is linked to MIC2 (lod 11.87 at 0.3). The lod scores testing linkage between DXYS14 and sex are strongly negative. Testing for linkage between the disease allele and the markers produced conclusively negative lod scores in all cases for the three affection models used in this study (Table 1). Multipoint lod scores computed for the three markers were strongly negative throughout the whole of the pseudoautosomal region. Heterogeneity testing did not suggest that there was a subset of families linked to this region. When the MIC2 analysis was repeated with the female:male distance ratio set to 10, to test for a locus on the other side of the pseudoautosomal boundary, the lod scores were also negative. When male and female recombination fractions were allowed to vary independently, the maximum lod scores obtained by ILINK were only weakly positive, and were no more positive than would be expected by chance. Extended sib pair

Table 1
Two-point lod scores between schizophrenia and markers at the DXYS14, MIC2 and sex for each of the affection models assuming dominant inheritance

θ	Recombination fraction						
	0.00	0.01	0.05	0.1	0.2	0.3	0.4
Model: DOMS							
DXYS14	−10.097	−8.924	−7.189	−5.936	−4.293	−3.056	−1.833
MIC2	−4.517	−4.221	−3.352	−2.635	−1.739	−1.160	−0.673
Sex	−6.493	−5.821	−4.036	−2.710	−1.203	−0.434	−0.083
Model: DOMSS							
DXYS14	−9.893	−8.984	−6.946	−5.374	−3.338	−1.900	−0.630
MIC2	−7.193	−6.460	−4.881	−3.756	−2.393	−1.507	−0.786
Sex	−5.363	−4.790	−3.326	−2.140	−0.743	−0.128	−0.027
Model: DOMSSF							
DXYS14	−8.225	−7.923	−6.888	−5.817	−4.102	−2.733	−1.463
MIC2	−7.250	−6.999	−6.107	−5.164	−3.643	−2.403	−1.240
Sex	−7.232	−6.788	−5.211	−3.632	−1.550	−0.493	−0.074

Table 2
Extended sib pair analysis (ESPA) using the DOMSS affection model with DXYS14 and MIC2

	Not shared	Shared	Lost	χ^2	P
		MIC2			
Completely known:	2.00	1.00	25.00	0.33	<0.2920
Missing parents:	22.00	25.29	64.71	0.23	<0.3270
Missing sib(s):	1.00	0.06	0.94	0.83	<0.1850
Total χ^2				0.04	NS
		DXYS14			
Completely known:	3.00	6.00	3.00	1.00	<0.1580
Missing parents:	30.00	37.03	14.97	0.74	<0.2010
Missing sib(s):	0.00	0.00	6.00		
Total χ^2				1.32	NS

analysis (ESPA) showed no significant excess of alleles shared between affected sibs (Table 2).

Discussion

The XY region can be a problematic chromosomal area to test for linkage to a disease locus because there is asymmetric crossing over (recombination) between a pair of X chromosomes at female meiosis compared to XY recombination in males. Because of asymmetry in recombination fraction in male and female meioses, it is possible that linkage of an XY locus to sex could confound linkage to a disease if there was some distortion of the sex concordance ratios. As some evidence of increased same-sex concordance for males has been found, and because these males must inherit the Y region from their fathers, it is possible that weak sex linkage of the DXYS14 marker could produce spurious evidence in favour of linkage for this locus in sib pair and classical linkage analysis. One way of finding out is to analyse maternal meioses on their own, since in these the maternal XY region is randomly and equally transmitted to male or female offspring. When this control has been carried out in the previous studies, evidence for pseudoautosomal linkage has been weakened. The MIC2 locus has a strong sex linkage and lod analysis must take this into account.

The same-sex concordance for schizophrenia that has sometimes been found may have arisen because of other factors such as increased penetrance in males or selection biases. Therefore the weakly positive lod scores reported by Crow *et al* (1994) are less significant than they might appear because they are derived from a sample which has previously been shown to have an excess of male/male sibling pairs both affected by schizophrenia (Crow, 1989; Collinge *et al*, 1991). This would artificially inflate the lod scores with markers linked to sex on the Y chromosome.

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In the present study, testing is carried out with three different markers and three affection models. The highly negative lod scores lead us to conclude that the XY region is probably not involved in any substantial proportion of the families that we have been studying in Iceland and the UK: nor is there any evidence to suggest that a locus further down on the sex chromosomes might be involved.

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